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The use of immunologic data for regulation of environmental pollutants is obviously in its infancy. Major factors that affect the usefulness of immunologic data for regulatory decisionmaking and describe an approach we are presently developing to answer questions related to immunotoxicity of chemicals are discussed in this paper.

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THE USE OF IMMUNOTOXICOLOGICAL DATA IN THE ENVIRONMENTAL REGULATORY PROCESS

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INTRODUCTION

One of the principal responsibilities of the U.S. Environmental Protection Agency is to conduct research necessary to maintain an adequate scientific and technological data base on which to establish the standards, regulations and implementation actions required by various congressional acts. We will focus this discussion on two legislative mandates; the Clean Air Act and the Toxic Substances Control Act, because most of our research related to immunotoxicity has been done to provide data for these two.

The Clean Air Act, as amended in 1977, authorizes a national program of research directed towards controlling adverse health effects of airborne chemicals. Research toward this end falls into two categories: that to support National Ambient Air Quality Standards (NAAQS) and that to identify, evaluate and control other hazardous air pollutants.

NAAQS have been set for ozone, nitrogen dioxide, sulfur dioxide, total suspended particulates, carbon monoxide, and lead. The goal is to set standards at levels which, in the judgment of the EPA Administrator, protect the health of normal and susceptible subpopulations with an adequate margin of safety. The scientific data bases used in setting these standards are updated and reviewed every five years. As scientific

information becomes available, uncertainties inherent in NAAQS are reduced, and reliance on judgmental factors becomes less important.

In addition to the chemicals controlled by NAAQS, there are a vast number of chemicals in the ambient air, some of which may be responsible for serious irreversible and/or incapacitating disease. Over the past several years, increasing attention has been directed towards the identification, evaluation, and control of these hazardous air pollutants (HAP) to which no NAAQS is applicable. Hence the EPA has been authorized to regulate mutagens, carcinogens, teratogens and other toxic pollutants. Seven compounds: benzene, asbestos, vinyl chloride, inorganic arsenic, radionuclides, mercury, and beryllium are now listed as hazardous. Of these, only the latter two are listed for non-carcinogenic reasons, and so far no chemical has been regulated on the basis of immunotoxicity.

The objectives of the health effects research being conducted to support these two environmental regulatory programs are quite different. Updating the relatively large data base to support the maintenance or revision of the NAAQS requires research to 1) better define dose-response relationships; 2) develop more information on sensitive population groups; 3) determine effects of pollutant interactions; 4) confirm and extend important findings of the data base; 5) examine mechanisms of action; 6) increase understanding of the degree of adversity of the observed health effects; and 7) develop exposure estimates for the general as well as the sensitive population. Essentially the data base is being refined. Major objectives of the HAP toxicology program are to 1) improve and apply identification and screening methods; 2) expand the data base for use in the assessment of potential risk; and 3) better define concentration-response relationship for predicting significant

levels of exposure. Thus, under this program, the data base is essentially being created.

The other legislative mandate, the Toxic Substances Control Act (TSCA) requires that EPA establish chemical testing guidelines for industry to use to assess the toxicity of the chemicals they produce. The research conducted to support this program differs from that which supports the air program in that it is done primarily to 1) refine and review existing research guidelines; 2) improve the data base for registration and regulations; and 3) identify methods for testing which can be used in qualified industrial and commercial laboratories. Immunotoxicity testing falls into this last category since at the present time it is not included in the toxicity testing guidelines.

The use of immunologic data for regulation of environmental pollutants is obviously in its infancy. In the following discussion we will consider major factors that affect the usefulness of immunologic data for regulatory decision making and describe an approach we are presently developing to answer questions related to immunotoxicity of chemicals.

USE OF IMMUNOLOGICAL DATA FOR REGULATORY PURPOSES

Almost any scientifically sound immunotoxicological study has some information useful for regulatory decision making. However, there can be a considerable range in the degree of usefulness of such studies. Major factors which influence regulatory relevance are discussed below.

Chemical to be tested: The chemical to be examined for immunotoxicity must be environmentally relevant. For those ubiquitous pollutants regulated by NAAQS, the identity of chemicals of interest is clear. The only significant exception is for particulate matter since it is a broad

class of chemicals primarily consisting of ammonium sulfate, carbonaceous particles, particles emitted by wood burning, sulfuric acid, alumino-silicate compounds, ferric oxide, and calcium carbonate.

Other chemicals of interest to EPA under the HAP regulatory authority are more difficult to identify. In this case the major problem is the multitude of chemicals, primarily metals and organics, and the paucity of data bases on environmental monitoring and health effects. Knowledge of production volumes, industrial waste volume, environmental persistence and dispersion tendencies in relationship to the size of populations being exposed provides guidance on selection of chemicals. However, information of this sort is often lacking. Also, it is conceivable that a highly toxic chemical might have low exposure rates, making it difficult to automatically conclude that only high volume chemicals are of interest. Obtaining health effects data is critical.

A major determinant of regulatory relevance is whether the chemical form being tested occurs in the environment to a substantial degree. For instance, the predominant form of metals in the air is as oxides, not soluble salts, yet most investigations use the salts. This can make an important difference as indicated by the following examples: Host defenses against bacterial pulmonary infection were more sensitive to $NiCl_2$ than to $NiSO_4$ and more sensitive to $ZnSO_4$ than $Zn(NH_4)_2(SO_4)_2$ (1,2), primary antibody responses were significantly reduced by $NiSO_4$ and $NiCl_2$ but not NiO (3); Pb_2O_3 and NiO caused more alterations in the number of alveolar macrophages in the lung than did $PbCl_2$ and $NiCl_2$ (4,5), and Waters et al. (6) found that the cytotoxicity of vanadium was related to the solubility, with the more soluble forms being more toxic.

The physical state of the chemical is also critical. For example, several inhalation studies of silica have been performed, but they used smaller particle sizes (< 3 μm, mass median aerodynamic diameter, MMAD) than typically exist in the ambient air (about 7 μm, MMAD). Because particle size influences the site of deposition in the respiratory tract and clearance (7,8), immunotoxic data developed on a particle size which is uncommon in the air may have unknown relevance to EPA. An even more complex issue is that of chemical interactions. Man is exposed to pollutant mixtures, but most toxicological studies are conducted with single chemicals. This issue requires more experimentation to increase our level of understanding. Many of the chemicals of interest to EPA are organics which undergo biotransformation. Since there are species differences in xenobiotic metabolism, it is conceivable that data developed in a rat might have limited applicability to man. However, the precise nature of such limits is unknown.

The foregoing indicates how previously conducted research has strengthened our understanding of the needs for relevance if data is to be used for regulation purposes. More basic research in this area will no doubt continue to sharpen our focus (in terms of relevance) and result in improved experimental protocols in the future.

Exposure protocol: The route of exposure can be a major determinant of immunotoxic potency. For example, Ni and Cd are more potent when administered by inhalation compared to intramuscularly (2). The time of exposure can also be critical. Following a prolonged exposure to an H_2SO_4 and carbon mixture and a mixture of SO_2 and carbon, there was immunoenhancement, followed by no change, followed by immunosuppression as exposure proceeded (9,10). Thus, misinterpretations of immunotoxicity could occur if examinations are only made at one time point in a study.

As with other toxicological studies, some immunological research is conducted using only 1 dose of the chemical. However, for the most part dose-response data are needed for regulatory decisions. Several groups are developing risk assessment and extrapolation (from animal to man) models which, when developed, will make dose response data even more crucial. There is also the issue of potency ranking of chemicals. In the future this might be used to relate the toxicity of an environmental chemical having no human data base to that of a chemical having both a human and animal data base.

Interpretability of the data: Regulators are faced with the problem of interpretating the data in terms of public health risk. For typical immunotoxicity data, however, there is no consensus. What is the significant risk to human health if a chemical causes a 30% statistically significant decrement in the number of IgM plaque forming cells in the spleen of mice? To answer this or similar questions will require major advancements in our understanding of immunotoxicity. First is the qualitative extrapolation. Is a 20% decrease in specific antibody titer of a mouse equivalent to a similar 20% drop in a man, and if so, will man experience an increased incidence or persistence of infection? Notwithstanding the differences between laboratory animals and man, there are extensive similarities, making it likely that if an effect occurs in a number of animal species, the same type of effect will occur in man. Thus, the greatest problem is the definition of degree of adversity of a particular experimental observation.

The second problem is the quantitative extrapolation from animals to men. If an effect occurs at a given dose of chemical in an experimental laboratory animal, what dose will cause the same effect in man.

With such knowledge, one could determine whether environmental levels were above or below this human effective dose and take the appropriate regulatory strategy. Quantitative extrapolation has two major components - dosimetry and species sensitivity (11). Briefly, dosimetry involves determining the relationship between inhaled concentration and regional pulmonary tissue delivered dose for several species, including man. Sensitivity is the determination of whether the same delivered tissue dose in many species will produce the same response in each of these species.

Another major issue in interpreting human risk to immunotoxic chemicals is host factors which may influence the outcome of exposure. The developing or senescent immune system may be more sensitive to chemical exposure than that of a normal young adult. Other sensitive subpopulations may exist. Risk factors in immunotoxicity have received relatively minor attention, making it difficult to interpret data to fashion regulatory strategies to protect the largest numbers of people.

Dissemination of data: A first major step in developing regulations for air pollutants is a detailed review of the literature. The review is subject to intense scrutiny. If the papers being reviewed are not complete and precise, the data contained will have little use. Papers are reviewed according to criteria which include nature of publication (peer-reviewed is preferred), adequacy of experimental design and statistics, and extent of description of methods and results. Many papers in the peer-reviewed literature lack details such as number of animals used, identification of statistical methods, specific identification of chemical levels (including generation and monitoring techniques, calibration of monitors and stability of exposure levels), and

specific design elements. For regulatory use, facts not in evidence in the literature reviews cannot be considered. Thus, a significant amount of information is lost because regulatory reviews are, by obvious necessity, rigorous.

AN APPROACH FOR IMMUNOTOXICITY TESTING

There are now a number of studies (12-16) which indicate that environmental chemicals depress various aspects of the immune response as determined by one or more of a myriad of tests available to assess the functional integrity of specific components. One of the problems which EPA faces in using such data for regulatory purposes is that few attempts have been made to correlate changes in immune responses with increased incidence or severity of disease. Because the Agency needs to be able to define effects in terms of their degree of adversity, we have taken an approach which is the reverse of that taken by many other investigators. We are developing a series of disease susceptibility models and are combining these with specific immune function tests to determine if any of the immune responses are depressed in animals exhibiting increased disease enhancement following exposure to a chemical.

Since more than one host defense mechanism is usually involved in protecting an individual from a given disease, (e.g. infectious, neoplastic, allergic, or autoimmune diseases) susceptibility models usually reflect the combined effects of a chemical on several immune defense mechanisms and their interactions. If the mechanisms of pathogenesis and host response to a particular disease are known, these provide clues as to the types of specific immune functions which might be affected in

animals exhibiting enhanced symptoms. Effects of chemicals on these mechanisms can then be tested using immune function tests and some assessment can be made as to the degree of immune suppression which is necessary to exasperbate symptoms. It is theoretically possible to develop for immunotoxicity testing purposes a series of disease susceptibility models, which taken together, may reflect the health of the entire host defense system.

The practicality of developing an immunotoxicity testing tier which utilizes disease enhancement as the first step in the tier has yet to be determined, but it is certain that no immunotoxicity tier would be valid without providing disease susceptibility models somewhere in the scheme. We have developed two such models which utilize infectious agents. One of these reflects pulmonary defense mechanisms and the other systemic (primarily cell-mediated) defense mechanisms.

For a number of years, we have used susceptibility of mice to an aerosol infection with the bacteria Streptococcus pyogenes Group C as an indicator of effects on pulmonary defense mechanisms (17-19). Briefly, animals are randomly selected to be exposed to either clean air or to the test substance. After the cessation of this exposure, the animals from both chambers are combined and are exposed to an aerosol of viable microorganisms. At the termination of this exposure, some animals from each group are sacrificed. The remainder of the animals are housed in clean air, and the rate of mortality in the two groups determined during a 15-day holding period. The control mortality is approximately 10-20% and reflects the natural resistance of the host to the infectious agent. Other parameters such as number of organisms per lung at different time periods can also be measured. This particular model has demonstrated

toxic effects of numerous gases and particles (NO_2, O_3, $CdCl_2$, $NiCl_2$, phosgene, toluene, etc.) which can be related to the duration of exposure at different concentrations (1,17,19, unpublished data).

If host defense mechanisms are functioning normally, there is rapid inactivation of inhaled bacteria that have been deposited at the alveolar level (20-21). However, in pollutant-exposed animals, the number of microbes in the lungs increases in number beyond the original number deposited within 4-6 hrs (22-24). This acceleration in bacterial growth has been attributed to the pollutant's alteration of the capability of the lungs to destroy bacteria, thus permitting those with pathogenic potential to multiply and produce disease.

A number of host defense mechanisms operate in the respiratory system. These include alveolar macrophages, secretory antibody, interferon, cytotoxic T lymphocytes and natural killer cells, mechanical barriers such as the mucociliary system, and the various components of the inflammatory system. The nature of this infectivity streptococcal model allows one to focus on the possible mechanisms for pollutant enhancement of disease to the following: 1) production of edema; 2) reduction in physical removal by mucociliary mechanisms; and/or 3) alteration of non-specific cellular defense mechanisms. A number of studies suggest that the effects of pollutants on the streptococcal infection are largely a result of adverse effects on the alveolar macrophage (25-28). Hence, for any pollutant tested which enhances this infection, the suspected primary mechanism of action is alveolar macrophage toxicity.

Another model which we are developing uses susceptibility of mice to murine cytomegalovirus as an indicator of effects on certain systemic defense mechanisms. Human cytomegalovirus is one of the several oppor-

tunistic infections which commonly cause disease in immunosuppressed individuals (29-31). For this reason, it is a good candidate for studies in which increased susceptibility to viral disease is u ed as an indicator of immunosuppression. Cytomegaloviruses (CMV) are species specific, so human CMV does not infect laboratory animals. However, murine CMV (MCMV) has been extensively developed as an experimental model (32-34). As in humans, MCMV in mice is usually a benign infection which can be greatly exacerbated by immunosuppression (35-37). Immune defense mechanisms thought to be important to host defenses against MCMV include natural killer (NK) cell activity (38,39), cytotoxic T cell activity (40), macrophage activity (41), and interferon (42). It happens that these same defense mechanisms are also prominently mentioned in tumor immunology (43). MCMV might, therefore, provide a disease susceptibility model which can be more rapidly assessed than increased tumor susceptibility but yet be predictive of the latter.

Experiments to date have shown that cyclophosphamide and $NiCl_2$ enhancement of acute MCMV can be correlated with inhibition of natural killer cell activity (44). With a third pollutant, parathion, mice dosed at the peak of MCMV infection of the liver also exhibited enhanced mortality; however, this occurred too soon after parathion treatment to be indicative of an altered immune response. Such a study indicates that health effects resulting from combined activity between infectious agents and chemicals do not always involve the immune system. While this is not, strictly speaking, an immunotoxicology problem, it is nevertheless important to consider. For instance, most of the theories advanced, which link Reyes syndrome to synergistic activity between toxic chemicals and virus, do not involve immunotoxicity but do involve

virus-chemical interactions which affect the liver (45,46). It is clear from this example that disease susceptibility models must be accompanied by immune as well as function tests in order to accurately evaluate immunotoxicity.

Obviously the two models we are using do not begin to represent all the host defense mechanisms that could be impaired by chemical exposure. They are merely illustrative of an approach which can be taken to identify substances which are immunotoxic. At the present time, immunotoxicity testing is not required under TSCA, and data from immune function tests and disease susceptibility models have not been used directly in regulating pollutant exposure.

A Case Study of Regulatory Use of Data: Epidemiological studies have been conducted in U.S. and Great Britain which indicate that the incidence of respiratory illness, bronchitis and cough is higher in children living in homes with gas stoves (47-50). Significantly higher levels of NO_2 in gas versus electric stove homes have been documented (51,52). In evaluating the evidence, major uncertainties exist regarding what specific agent(s) caused the response and were associated with this effect.

Experimental work with the animal infectivity model has provided sufficient data to demonstrate that NO_2 impairs respiratory defense mechanisms. The findings of these studies suggest that (1) short-term peak exposures may be more important than long-term, low level exposure of equivalent dose (CxT); (2) background concentrations may affect the ability of the host to recover from peak exposures, and (3) that intermittent exposures may not produce an effect that is significantly less than a similar continuous exposure (53-56). Because of the available

data, it was reasonable to suggest that the peaks of NO_2 may be the factor for increased prevalence of respiratory illness for young children living in homes with gas stoves. Thus, the animal data from the infectivity model provided the toxicological plausibility behind the statistically significant associations observed in the epidemiological study, thereby facilitating use of the entire set of data in decision making relative to the review of the NAAQS for NO_2.

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